Symbiotic relationships between silverfish (Zygentoma: Lepismatidae, Nicoletiidae) and ants (Hymenoptera: Formicidae) in the Western Palaearctic. A quantitative analysis of data from Spain

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Abstract

A large dataset of various associations between silverfish (order Zygentoma) and Formicidae is presented; this was obtained from samples collected across continental Spain. Associations have been detected in 693 ant nests of 14 different genera of Formicidae, hosting two species of Zygentoma belonging to the family Nicoletiidae (subfamily Atelurinae) and 17 species of Lepismatidae (subfamily Lepismatinae). A high diversity of interactions has been found: Overall, 157 different associations (species of Zygentoma – species of Formicidae) have been recorded. Comparing our data with the existent literature, 41 of these pairs are reported here for the first time.

A quantitative criterion is being followed to classify taxa of Spanish Zygentoma occurring in ant nests. According to their obligateness, three groups are distinguished: xenomyrmecophiles, occasional and strict myrmecophiles and, in the latter, at least two degrees of host specificity: generalist and specialist species. A cladogram of Spanish Lepismatinae places specialist silverfish as the more apomorphic taxa.

Moreover, the number and type of guest species of the most frequent ant genera and the number of individuals and species per nest have been compared and the Zygentoma-Formicidae quantitative network has been analysed. In conclusion, more than one mode of association occurs between Zygentoma and Formicidae in the Western Palaearctic. Nests of *Messor* FOREL, 1890 host a lot of species of silverfish, most of which are specialists that have likely developed a higher level of integration and are far from strict parasites. A second group of associations is represented by several common ant genera such as *Camponotus* MAYR, 1861, or *Formica* LINNAEUS, 1758, which mostly host a few species of myrmecophilous Zygentoma (those that are considered generalists). The position of *Aphaenogaster* MAYR, 1853, is intermediate between these two opposite groups. In the latter two groups, silverfish are likely parasites.

Key words: Zygentoma, Lepismatidae, Thysanura, Formicidae, myrmecophiles, symbiotic relationships, Western Palaearctic, Spain.

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Introduction

It is generally known that several species of silverfish (Zygentoma = Thysanura s. str.) live in association with ant colonies (WASMANN 1894, WHEELER 1910, WILSON 1975). Already in the 19th century, the silverfish-ant association was documented; *Atelura formicaria* HEYDEN, 1855 was the first species of myrmecophilous Zygentoma that was described on the basis of specimens that were found with ants, specifically with *Tetramorium caespitum* (LINNAEUS, 1758).

Two families of silverfish include myrmecophilous species: Nicoletiidae and Lepismatidae. Inside the first one, only species of the subfamily Atelurinae are usually associated with ants or termites (PACLT 1963, KISTNER 1982). Compared with tropical regions, the diversity of Atelurinae in the Palaearctic is very poor; only two genera are widespread: *Atelura* HEYDEN, 1855 and *Proatelurina* PACLT, 1963, both of them represented by one species in Spain. On the other hand, several species of Lepismatidae are reported to live with ants (PACLT 1967, MENDES 1987), most of them belonging to three subfamilies: Mirolepismatinae, Acrotelsatinae and Lepismatinae. The first is centred in America and two of its genera (*Prolepismina*

Tab. 1: Classification of Zygentoma in terms of their association with ants. Thw groups in MENDES (1987) are compared to the groups that are established in this work using quantitative criteria. The criteria of our classification are given in the Table, with the criteria of Mendes being described in the text.

Mendes' groups	New groups	Quantitative criterion used	Biological characteristics of the group
a. Xenomyrmecophiles	A. Xenomyrmecophiles	The absence of association with ants is significant in a binomial test.	These species live in different habitats but they are found in ant nests rarely.
	B. Occasional (or facultative) myrmecophiles	Both absence and presence with ants are not significant in a binomial test.	This group includes species with a more marked tendency to live inside ant nests, but they can be found often without relation with ants. When living with ants, they usually do not show preference for one ant genus (they can be considered as generalists).
	C. Strict myrmecophiles (=Myrmecophiles)	The association with ants is significant in a binomial test.	These species live usually inside ant nests and develop ethologic and morphologic adaptations to live with ants.
b. Panmyrmecophiles	C.1. Generalists	The percentage of associations with any genus of ants is less than 70% of the total interactions that are registered for the species.	These species are strict myrmecophiles, but do not show a marked preference for one genus of ants; they live with many ant genera (but not with all of them, as suggested by the denomination "panmyrmecophiles").
c. Symphiles	C.2. Specialists	The percentage of associations with only one genus of ants is greater than 70% of the total associations that are registered for the species.	These species are strict myrmecophiles, show a clear preference for a genus of ants, and live always or nearly always in the nests of one genus. The term "Symphiles" is not adequate because, according to Wasmann's criteria (WASMANN 1894), it implies a concrete type of association that does not match with the silverfish establishment with ants.

Fig. 1: An interaction of a *Messor structor* worker facing the silverfish *Neoasterolepisma spectabilis*, a frequent guest in *Messor* nests, with no evidence of aggressive behaviour by the ant. Photograph by R. Molero-Baltanás, obtained in Lucena (Córdoba), Spain.

SILVESTRI, 1940 and *Mirolepisma* SILVESTRI, 1938) are usually found with ants. This is also the case of the deserticolous genus *Lepismina* GERVAIS, 1844 (subfamily Acrotelsatinae). However, most references in the literature correspond to Lepismatinae, with more than 30 species of myrmecophiles. Lepismatinae associated with ants have been found in the Afrotropical Region and in the poorly studied Oriental and East Palaearctic Regions (MENDES 1988); they are well represented in the Mediterranean basin and are particularly diverse in Spain, with 18 species in the

Iberian Peninsula (MOLERO-BALTANÁS & al. 2002). Because the relationship of Lepismatinae with ants has been poorly studied, this paper gives a greater emphasis on this group (Fig. 1).

The information provided in the literature about the biological aspects of the relationship between ants and silverfish is scant. A lot of species are rarely reported with ants, and in these cases, nothing can be concluded. With the scarce data available, MENDES (1987) established a classification of Zygentoma species depending on their obligateness and host specificity, distinguishing three groups of Zygentoma: "Xenomyrmécophiles" (or "Myrmecoxenes", i.e., usually absent from ant nests), "Panmyrmécophiles" or "Myrmécophiles s.l." (i.e., usually found with ants belonging to any taxon) and "Symphiles". Inside the latter group, which includes host-specific species, Mendes established several subdivisions according to the ant group with which they are associated. This classification was based on qualitative considerations and supported by a quite limited number of samples and observations. Although derived from Mendes' classification, the division proposed in this work is based on quantitative criteria and distinguishes the following groups: true xenomyrmecophiles, occasional myrmecophiles and strict myrmecophiles; inside this last group, there are generalist and specialist species. The correspondence between Mendes' groups and ours and the criteria for this classification are presented in Table 1.

From the point of view of the type of these associations, several classifications have been proposed, such as the classical division made by WASMANN (1894) and followed by BERNARD (1968) or those presented by WILSON (1975) and HÖLLDOBLER & WILSON (1990). Summarising the categories presented by these authors, we can distin-

guish mutualism, in which both members benefit, from antagonistic relationships (predation or parasitism). An intermediate category, commensalism, can be created for those guests that do not harm or benefit the ants. However, some associations do not fit completely in any of the categories and a special one must be created for a particular relationship (LE MASNE 1994).

Concerning the biology of myrmecophilous Zygentoma, most of the information found in the literature is restricted to the taxon of the ants which silverfish are associated with. The scarce concrete information available had led to some authors considering silverfish to be either parasites or commensals.

The option of parasitism is supported by the observations of JANET (1896) and WITTE & al. (2009), based on species of the subfamily Atelurinae: the European *Atelura formicaria* with *Lasius umbratus* (NYLANDER, 1846) and the Malaysian *Malayatelura ponerophila* MENDES, VON BEEREN & WITTE, 2011, with *Leptogenys distinguenda* (EMERY, 1887). Both authors reported that these silverfish rob the ants of part of the nutritional drops that they exchange and avoid the aggression of ants by escaping quickly (*Atelura*) or by using imperfect chemical camouflage (*Malayatelura*).

In contrast, the observations of RETTENMEYER (1963) on another Atelurinae, the American *Trichatelura manni* (CAUDELL, 1925) with *Eciton* spp. LATREILLE, 1804, suggest that at least in some cases, some silverfish could behave as commensals or even provide some services to the colony. WILSON (1975) considered that silverfish would be considered commensals, designating their relationship "nest commensalism". Moreover, SILVESTRI (1912), PACLT (1956), BERNARD (1968) and MENDES (1987) agree with this hypothesis and even the possibility of emerging mutualistic associations, but do not support this with experimental evidence, so antagonism is currently assumed as the most likely option "by default".

As each of the aforementioned authors proposed only one type of relationship, none of them raised the possibility that, as myrmecophilous Zygentoma and ants are diverse groups with diverse biology, there could be different modes of association. However, during a sampling of silverfish fauna performed across the Spanish territory (carried out mainly during the period 1986 - 1994 for the "Fauna Ibérica" Project), we noticed that this hypothesis could be confirmed. An important dataset of all the myrmecophilous silverfish of the Spanish fauna was obtained, allowing us to perform an extensive analysis. The first target of this analysis is identifying which Zygentoma-Formicidae associations occur in Spain and compare this information with previously published data to determine which ones are reported for the first time. After this, we aim to classify Zygentoma species in relation to their association with ants (degree of myrmecophily and host specificity) on the basis of a quantitative criterion, distinguishing between occasional and strict myrmecophiles and, within this group, to identify generalist and specialist taxa. We also intend to check whether these groups correspond with monophyletic clusters in order to glimpse how myrmecophily evolved inside West-Palaearctic Lepismatinae. Moreover, the quantitative analysis of this dataset is used to discriminate whether all the ant-silverfish associations in Spain are similar (all considered as parasitism?) or if there is more than one mode of interaction (interpreted as commensalism or mutualism?). Presenting and analysing the network pattern of the associations between Zygentoma and ants in Spain can clarify the aforementioned question and provide a network pattern that can be compared with other ecological networks.

Material and methods

Three groups of data are considered in this work. The first group comes from the literature referring to silverfish-ants associations in the West-Palaearctic region, including 225 reports. Only those works performed by Zygentoma specialists have been considered (a lot of misidentifications have been detected in the remaining papers). A list of these works is presented in the references section of Appendix S1 (as digital supplementary material to this article, at the journal's web pages); from these, we have accounted only those reports where ants were identified. These reports have not been used to add their data to our quantitative analysis because they are not comparable in terms of sampling methods; however, they are used to support our conclusions.

A second group of data includes published data from our own samplings in continental Spain and the Balearic Islands (reported in papers detailed in Tabs. S1.1 - S1.73 in Appendix S1).

Finally, a third group includes unpublished data from our own samplings in the same territory; this subset of data comes from an unpublished PhD thesis by one of the authors (R.M.B.) and are treated here as new data (and detailed in Appendix S1). The two latter groups of data are now joined together to form a dataset that is used for a quantitative analysis.

The three groups of data are considered together in Appendix S1 (Tabs. S1.1 to S1.73) and in some parts of the Discussion section to generalise our conclusions.

Our sampling scheme includes more than 1000 localities and several thousands of ant colonies, homogenously covering all regions of Spain; for a map, see MOLERO-BALTANÁS & al. (2002). The sampling was carried out to identify all of the taxa of silverfish of the Spanish fauna from all of the habitats where they can occur. For this reason, all types of habitats were examined, including ant nests. Silverfish were mostly found under stones, but also in vegetal debris, trunks of trees, human dwellings, caves, soil, etc. Habitats with different ecological conditions and different anthropogenic modification intensities were inspected (urban environments, agroecosystems, autochthonous oak-trees and introduced pine forests, semiarid shrubland, plains, mountains, etc.). All of the sampling was performed by the same individual, taking the same time (1 hour) in each locality; although performed in all seasons of the year, the experience suggested concentrating the efforts in the Northern parts of Spain during the period from May to September.

Most samples were obtained more than 20 years ago, but the ecological conditions have not significantly changed (R. Molero-Baltanás, unpubl.) and, with regard to the aims of this work, the results of the samples are representative of the present situation.

In each site, myrmecophilous silverfish were usually found under stones that cover ant nests and were caught with an aspirator together with some worker ants of the same nest. When the number of silverfish was high, it was

Tab. 2: Detailed data registered in Spain on the Zygentoma-Formicidae association. Data correspond to the number of nests of each genus of ants where each species of Zygentoma was found (834 associations in 677 nests where silverfish were identified at species level are accounted). This is a synthesis of Table S2.1 given in Appendix S2, after grouping data of each genus of ants. Acronyms of Zygentoma species see Table 4. Abbreviations of ant genera: Aph: *Aphaenogaster*. Bot: *Bothriomyrmex*. Cam: *Camponotus*. Cre: *Crematogaster*. For: *Formica*. Las: *Lasius*. Lin: *Linepithema*. Mes: *Messor*. Phe: *Pheidole*. Pla: *Plagiolepis*. Tap: *Tapinoma*. Tem: *Temnothorax*. Tet: *Tetramorium*. N: Total number of associations for each row (ant genus) or column (Zygentoma species).

		Zygentoma																		
		Av	Pp	Lb	Lc	Ls	Nb	Ncr	Ncu	Nd	Nf	Ng	Nh	Nl	Np	Nso	Nsp	Nw	Ta	Ti	N
Formicidae	Aph	2	18	1	1	2	0	1	19	6	1	0	13	1	6	0	3	3	0	0	77
	Bot	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	Cam	2	37	0	1	0	0	1	37	1	0	0	1	2	3	0	2	5	0	2	94
	Cat	0	1	0	0	0	0	0	11	0	0	0	0	0	0	0	1	0	0	0	13
	Cre	0	0	3	4	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	8
	For	0	9	0	0	0	0	0	29	0	0	0	0	2	2	0	0	0	0	0	42
	Las	1	14	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1	0	0	19
	Lin	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
	Mes	2	49	0	1	1	7	53	35	1	41	14	0	57	1	12	143	40	4	0	461
	Phe	0	44	2	9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	56
	Pla	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Tap	0	6	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
	Tem	0	2	2	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	6
	Tet	1	26	3	11	2	0	0	0	0	0	0	0	0	0	0	0	1	1	0	45
	N	8	211	11	29	6	7	55	132	8	42	14	15	64	13	12	150	50	5	2	834

not possible to collect all of the specimens from the nest, so the total number of hosted Zygentoma was not estimated. However, it can be considered that the sampling was homogeneous and the obtained data are comparable because all of the samples were obtained in the same way: In each case, the number of insects that were caught was the maximum possible within the constraints. Apart from the 693 nests where Zygentoma were found, many additional colonies were investigated, but data did not account for those where silverfish were not detected. From the aforementioned 693 nests, 16 were discarded for some analysis because Zygentoma could not be identified at species level.

An independent sampling where all ant nests were accounted was designed to test whether the ants preferred by silverfish are usually the most abundant or not. Initial results from eight sampling sites are shown in Appendix S2. This information suggests that silverfish prefer several genera of ants, and supports our sampling method: Since there is no evident relation between the number of nests of an ant genus and the number of these nests inhabited by silverfish, it is not necessary for our aims to account the total number of nests without Zygentoma. We have made the reasonable assumption that most frequent ant taxa (grouped by genus) are abundant enough in most localities to be available for silverfish, and these have the choice, influenced by their requirements and by the biology of the different types of ants, but not by the relative frequency of these types.

More than one species of silverfish cohabited in a significant proportion of the sampled nests where silverfish were found; this fact (known as parabiosis) has led each Zygentoma-ant couple to be considered a different association. For example, if three species of Lepismatidae were collected in the same ant nest, this situation was treated as one sample but with three different associations. As an illustrative example, 320 nests of the genus *Messor* hosted silverfish, but 461 associations were registered. The samples in which it was not possible to identify the Zygentoma at the species level were not included in Tables 2 and S2.1 (see Appendix S2), but they were considered in Table 5 and the subsequent analyses.

The identification of silverfish was carried out following the keys of MENDES (1988) and papers on taxonomic problems solved after publication of these keys (these papers, detailed in Table S1.73 in Appendix S1, established some new synonyms or even some new taxa). The identification of ants was based on the use of Collingwood's keys (COLLINGWOOD 1979, COLLINGWOOD & PRINCE 1998) and also the experience of one of the authors (A.T.). The studied material is deposited in the Departamento de Zoología, University of Córdoba, with the exception of the types of the new species of silverfish, which are deposited in the Museo Nacional de Ciencias Naturales (MNCN) in Madrid.

Once the ants and silverfish were identified, several statistical analyses were performed. Formicidae species were cross-tabulated together with the Zygentoma species hosted by them (see Table S2.1 in Appendix S2) and from this contingency table, a correspondence analysis was carried out using the ca package in R developed by NENADIĆ & GREENACRE (2007). To avoid the abundance of zeros in the contingency table, a similar analysis was performed after considering the genera instead of ant species (Tab. 2). More reasons to support why Formicidae species were grouped by genus are provided in Appendix S2.

To determine the fidelity of silverfish, Bonferroni corrected binomial tests were carried out for each genus and for each species of Zygentoma occurring in Spain; these tests allow the distinction between xenomyrmecophile taxa and those that can be considered occasional or strict myrmecophiles, particularly when the number of samples is sufficiently high. When the number of samples was not high enough (few infrequent taxa or data from the literature), we used the criterion to consider a species a xenomyrmecophile if ants were found in less than 10% of the samples, and a strict myrmecophile if more than 75% of the samples came from ant nests. Inside strict myrmecophiles, generalist and specialist species were separated following the criterion presented in Table 1.

With the intention to test if these groups are congruent with the evolutionary relationships of silverfish, a cladogram based on 24 morphologic characters (details of these characters and their states are provided in Appendix S3) was constructed with 20 species of Spanish Lepismatinae, using a xenomyrmecophile species as an outgroup. We decided to include species from the Canary Islands since they are well known by the authors and may help clarifying the evolutionary issues of this group in the Western Palaearctic region. Atelurinae were excluded from this analysis because this subfamily has an independent evolutionary origin and is poorly diversified in the studied region. Mesquite (MADDISON & MADDISON 2014) was the program used for this aim.

To compare the tolerance of different ant genera, several parameters of the associations were tested: Pearson chi-square tests and Bonferroni correction for the contrast of the difference of proportions and General Linear Model with Bonferroni multiple comparisons test to compare the mean number of silverfish per ant nest.

Program R (R CORE TEAM 2015) was used to perform all statistical tests and make related graphics. The bipartite package in R (DORMANN & al. 2008) was also used to obtain a plot of the bipartite network Zygentoma-Formicidae in continental Spain and calculate some parameters of this network, as well as some specialisation indexes of individual species.

Results

New data of the relationships between Zygentoma and ants in Spain. Results of the new samplings compared with existing literature

In our samplings in Spain from 1986 - 1994, 693 ant nests of 40 species belonging to 15 different genera of Formicidae (Fig. 2) were found, hosting 22 different species of Zygentoma: 2 Atelurinae, 19 Lepismatinae and 1 Coletiniinae, approximately 50% of the known taxa of this order in Spain (Tab. 2 and S2.1 in Appendix S2). Parabiosis has been detected in about 19% of the sampled nests where silverfish were found. The total number of associations of Zygentoma with ants is 834. During these samplings, more than 1000 samples of Zygentoma were gathered in other habitats, without any relation with ants (and in more than 4000 ant colonies, silverfish were not found).

Table S2.1 (Appendix S2) presents the ant-Zygentoma associations that were detected in our samplings, and Tables S1.1 to S1.72 (Appendix S1) compare these data with those documented in the literature, indicating those rela-
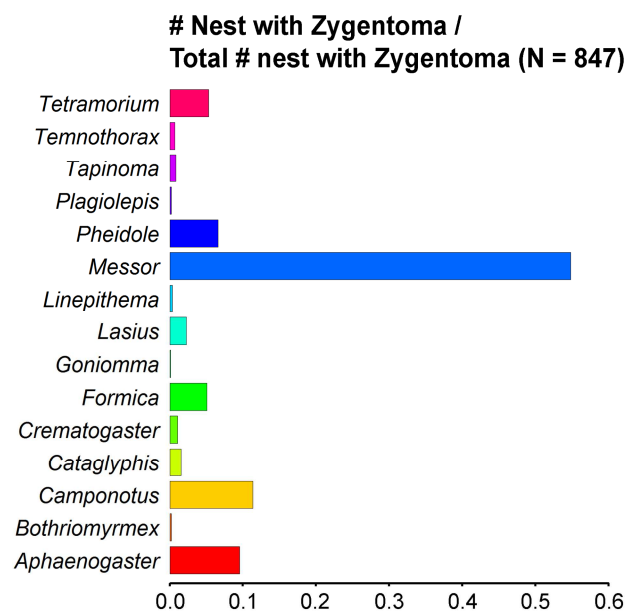

 # Nest with Zygentoma / Total # nest with Zygentoma (N = 847)

Fig. 2: Bar chart showing the frequencies of samples of the different genera of Formicidae hosting Zygentoma in Spain (these frequencies are relative to total number of nests with Zygentoma; nests without silverfish were not accounted).

tionships that are new (reported for the first time). Moreover, all of the data of the associations that have not been previously published are detailed in Appendix S1. In the Western Palaearctic, at least 51 species of 17 genera of ants have been detected, hosting at least 26 species of Zygentoma. A total of 193 different associations are reported; overall, 152 were previously known and 41 are cited for the first time. Moreover, 116 of the previously known relationships have been confirmed by our samplings in Spain.

Table 2 (and S2.1 in Appendix S2) also show that the greater proportion of associations corresponds to Lepismatinae of the genus *Neoasterolepisma* and to ants of the genus *Messor*. The number of associations of *Proatelurina* is also notable, within the Atelurinae. Table 2, grouping ant taxa by genus, is used for most of the subsequent analyses.

Classification of Spanish species of Zygentoma according to their fidelity to ants

Genus level

In Table 3, the number of samples of each genus of Spanish Zygentoma that were found with and without Formicidae is detailed. Using binomial tests with Bonferroni correction, we conclude that the genera *Neoasterolepisma* and *Tricholepisma* PACLT, 1967, as well as the two of Atelurinae, are strict myrmecophiles; the remaining genera can be considered xenomyrmecophiles.

Species level

Table 4 includes the classification of most Spanish species in the categories mentioned in the introduction and justified in Table 1. Some silverfish of the different groups proposed are illustrated in Figures 3 - 7 and a diagram with three bar charts of the most representative groups of strict myrmecophiles is presented (Fig. 8) showing their frequencies of occurrence with different ant genera. This

Figs. 3 - 7: Five species of Spanish silverfish classified in different categories according with their relationship with ants: (3) *Allacrotelsa kraepelini*, a xenomyrmecophile. (4) *Lepisma baetica*, an occasional myrmecophile. (5) *Proatelurina pseudolepisma*, a generalist. (6) *Neoasterolepisma delator*, an *Aphaenogaster* specialist. (7) *Neoasterolepisma lusitana*, a *Messor* specialist. Body lengths of specimens ranging from 5.5 mm in (4) and (5) to 10 mm in (3). Most silverfish species are not distinguishable without microscopic study.

Tab. 3: Data for the classification of Zygentoma genera of the Spanish fauna according to their association with ants. Results of the Binomial test with Bonferroni multiple comparison correction for each genus. The three last lines also detail the results for each species of the genus *Lepisma*. NS: number of samples that were studied for each genus of Zygentoma. WF: number of samples that were found with Formicidae. NF: number of samples without ants (found in other habitats). %WF: percentage of samples with ants with respect to the total of samples of Zygentoma of each genus. %NF: percentage of samples without ants. z: probability value as calculated by the binomial test. Sig: significance level associated with the z parameter. Explanation of the results in the text.

Zygentoma genera / *Lepisma* species	NS	WF	NF	%WF	%NF	z	Sig	Classification
Atelura	9	8	1	88.89	11.11			Strict myrmecophile
Proatelurina	234	210	24	89.74	10.26	12.16	<1E-10	Strict myrmecophile
Allacrotelsa	38	1	37	2.63	97.37	-5.84	1.14E-09	Xenomyrmecophile
Ctenolepisma	813	2	811	0.25	99.75	-28.37	<1E-10	Xenomyrmecophile
Neoasterolepisma	612	580	32	94.77	5.23	22.15	<1E-10	Strict myrmecophile
Tricholepisma	7	7	0	100.00	0.00			Strict myrmecophile
Coletinia	5	1	4	20.00	80.00			Xenomyrmecophile or Occasional myrmecophile
Lepisma	195	46	149	23.59	76.41	-7.38	<1E-10	Xenomyrmecophile
L. baetica	30	11	19	36.67	63.33	-1.46	0.30	Occasional myrmecophile
L. chlorosoma	57	29	28	50.88	49.12	0.13	1.00	Occasional myrmecophile
L. saccharina	108	6	102	5.56	94.44	-9.24	<1E-10	Xenomyrmecophile

Tab. 4: Silverfish species analysed and their acronyms used in this work. The column "myrmecophile category" assigns a classification for each species. Genus degree is the number of genera of ants linked with each species (the number in brackets indicates the number of genera where the association was observed more than once). SSI: species specificity index; d': weighted specialisation index for individual species. Species where these indexes are not calculated are those that are not included in the network because they are not Iberian (marked with *), they are xenomyrmecophiles or the number of available data is very low (? in the classification column). Authors of species that are not mentioned in the text are also indicated.

Species	Acronym	Myrmecophile category	Genus degree	SSI	d'
Allacrotelsa kraepelini (ESCHERICH, 1905)	–	Xenomyrmecophile	–	–	–
Atelura valenciana MOLERO-BALTANÁS, GAJU-RICART, BACH DE ROCA & MENDES, 1998	Av	Generalist	5 (3)	0.398	0.086
Lepisma baetica MOLERO-BALTANÁS, GAJU-RICART, BACH DE ROCA & MENDES, 1994	Lb	Occasional	5 (4)	0.404	0.478
Lepisma chlorosoma LUCAS, 1846	Lc	Occasional	8 (3)	0.457	0.454
Lepisma saccharina LINNAEUS, 1758	Ls	Xenomyrmecophile	–	–	–
Neoasterolepisma balearica MOLERO, BACH & GAJU, 1997	Nb	*Messor* specialist	1 (1)	1.000	0.081
Neoasterolepisma crassipes (ESCHERICH, 1905)	Ncr	*Messor* specialist	3 (1)	0.961	0.167
Neoasterolepisma curtiseta MENDES, 1988	Ncu	Generalist	6 (5)	0.406	0.312
Neoasterolepisma delator MOLERO-BALTANÁS, BACH DE ROCA & GAJU-RICART, 1996	Nd	*Aphaenogaster* specialist	3 (1)	0.750	0.272
Neoasterolepisma foreli (MONIEZ, 1894)	Nf	*Messor* specialist	2 (1)	0.975	0.166
Neoasterolepisma gauthieri ssp. *calva* MOLERO, MENDES, GAJU & BACH, 1994	Ng	*Messor* specialist	1 (1)	1.000	0.127
Neoasterolepisma hesperica MOLERO-BALTANÁS, BACH DE ROCA & GAJU-RICART, 1996	Nh	*Aphaenogaster* specialist	3 (1)	0.869	0.494
Neoasterolepisma inexpectata MENDES, MOLERO-BALTANÁS, BACH DE ROCA & GAJU-RICART, 1993	–	Xenomyrmecophile / Occasional?	–	–	–
Neoasterolepisma lusitana (WYGODZINSKY, 1941)	Nl	*Messor* specialist	6 (3)	0.883	0.131
Neoasterolepisma myrmecobia (SILVESTRI, 1908)	–	Occasional?	–		–
Neoasterolepisma pallida MOLERO-BALTANÁS, GAJU-RICART & BACH DE ROCA, 1995	Np	Generalist?	5 (3)	0.498	0.208
Neoasterolepisma soerenseni (SILVESTRI, 1908)	Nso	*Messor* specialist	1 (1)	1.000	0.120
Neoasterolepisma spectabilis (WYGODZINSKY, 1945)	Nsp	*Messor* specialist	5 (3)	0.950	0.262
Neoasterolepisma vulcana MENDES, BACH DE ROCA & GAJU-RICART, 1993	–	Xenomyrmecophile / Occasional?	–		
Neoasterolepisma wasmanni (MONIEZ, 1894)	Nw	*Messor* specialist	5 (3)	0.792	0.074
Proatelurina pseudolepisma (GRASSI & ROVELLI, 1890)	Pp	Generalist	13 (10)	0.307	0.241
Tricholepisma aurea (DUFOUR, 1831)	Ta	*Messor* specialist	2 (1)	0.810	0.050
Tricholepisma indalica MOLERO-BALTANÁS, BACH DE ROCA & GAJU-RICART, 1995	Ti	*Camponotus* specialist?	–	–	–

classification is also supported by binomial tests, correspondence analyses (Fig. 9) and the criterion to distinguish generalist from specialist species agrees with the species specificity index (SSI) calculated with the R bipartite package (DORMANN & al. 2008).

Occasional myrmecophiles. Binomial tests carried out at the species level gave similar results to those for silverfish genera, except for the genus *Lepisma* LINNAEUS, 1758. When species of this genus are studied separately (as shown in the three last lines of Tab. 3), heterogeneous trends can be detected: whereas *L. saccharina* LINNAEUS, 1758, shows a low percentage of association with ants (5.5%), *L. baetica* MOLERO-BALTANÁS, GAJU-RICART, BACH DE ROCA & MENDES, 1994, and particularly *L. chlorosoma* LUCAS, 1846, are associated with higher frequencies (36.7 and 52.5%, respectively). It can be con-
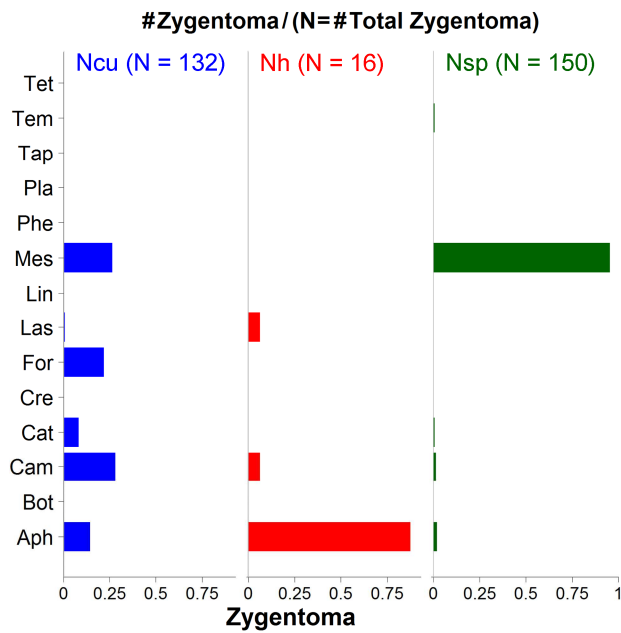
#Zygentoma / (N=#Total Zygentoma)

Fig. 8: Bar chart representing the proportions of association of three species of strict myrmecophiles, *Neoasterolepisma curtiseta* (Ncu, a generalist species), *N. hesperica* (Nh, an *Aphaenogaster* specialist), and *N. spectabilis* (Nsp, a *Messor* specialist), with different genera of ants. Proportions are relative to the total number of ant nests where each silverfish species has been found and not to the total number of nests, since this number is not the total of colonies sampled, but only the number of nests hosting silverfish. Moreover, these proportions show the generalist or specialist condition better.
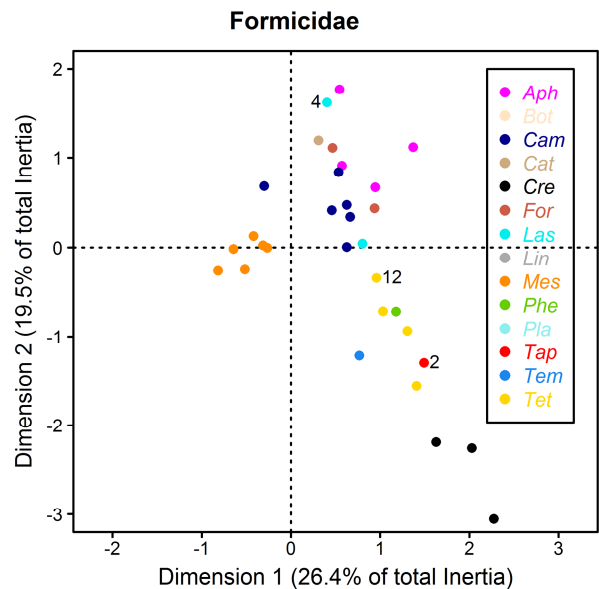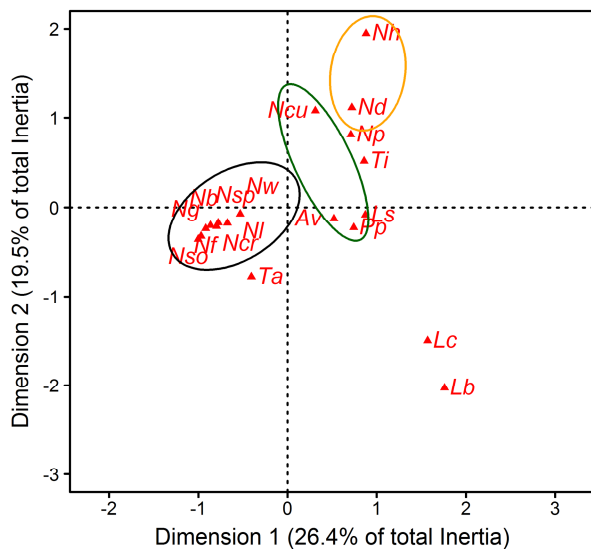

Fig. 9: Left: Plot representing the placement by the correspondence analysis of the different Zygentoma species of the Spanish mainland (acronyms: see Table 4). Groups that the analysis indicates are surrounded with lines: Group 1: *Messor* specialists (black line). Group 2: *Aphaenogaster* specialists (yellow line). Group 3: Generalists (green line). Some occasional myrmecophiles are placed in the bottom right-hand corner. The horizontal axe discriminates *Messor* specialists from the remaining genera and the vertical one discriminates from strict myrmecophiles and occasional species. Right: Plot representing the placement by the correspondence analysis of the different genera and species of ants hosting silverfish. Numbers in the plot indicate the coincidence of several species in the same place. *Messor* ants form a group nearly in the centre of the plot, while the remaining taxa are distributed along the Dimension 2 line of the plot (genera with wider tolerance are placed in the upper part of this line). The axes of both plots can be superimposed to show correspondence with the placements of silverfish species (for example, *Messor* ants are placed in a similar region of the plot as *Messor* specialists). The vertical axe discriminates ants with higher range and frequences of guests (in the top of the map) from those ant taxa with few guests and lower range of silverfish.

cluded that although *L. saccharina* is clearly a xenomyrmecophile, *L. chlorosoma* and *L. baetica* can be classified as occasional myrmecophiles. Therefore, we have included *Lepisma* in the subsequent analyses.

Generalist silverfish. Within strict myrmecophiles, two species of the Iberian fauna can be clearly considered generalists: the Atelurinae *Proatelurina pseudolepisma* (GRASSI & ROVELLI, 1890) and the Lepismatinae *Neoasterolepisma curtiseta* MENDES, 1988 (Fig. 8). The bar chart

of preferences of *P. pseudolepisma* (Fig. 11 in MOLERO-BALTANÁS & al. 1998) shows that it can be found with at least 13 different genera of ants. *Neoasterolepisma curtiseta* also inhabits nests of several genera of ants with a high frequency and there is no evident preference for one ant genus.

We can also include *Atelura valenciana* MOLERO-BALTANÁS, GAJU-RICART, BACH DE ROCA & MENDES, 1998 in the group of generalists, but without such a clear statist-
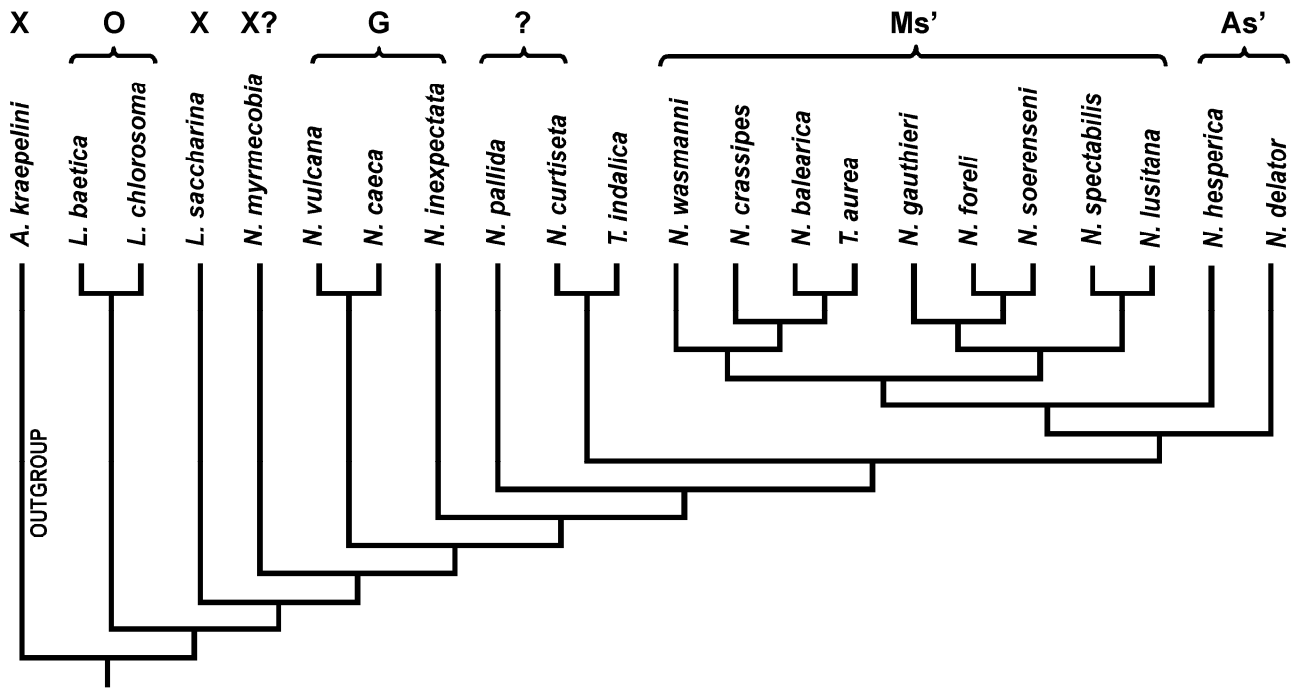
Fig. 10: Cladogram of Spanish Lepismatinae (including species from the Canary Islands and Continental Spain). The morphological characters and their status as considered for the analysis are presented in Appendix S3. X: xenomyrmecophiles. O: occasional myrmecophiles. G: generalist species. As': *Aphaenogaster*-specialists. Ms': *Messor* specialists.

Tab. 5: Data for the comparison of the number of specimens per nest and of the number of species of Zygentoma that were found with different genera of Formicidae. The genera that are marked with (**) are those that have been statistically compared (results in Table 6). Metrics given in columns are self-explanatory. Abbreviations: N.: number; Zyg.: Zygentoma; sp. / spp: species. The number given in brackets in the column of number of species hosted in Spain correspond to the number of additional species in Western Palaearctic.

Ant genera	N. of studied nests	% nests with respect to total N. of nests	N. of spp. hosted in Spain	Total N. of Zyg. specimens found in nests	Mean of N. of Zyg. specimens per nest	Variance of the N. of Zyg. specimens per nest	N. of nests with more than 1 sp. of Zyg.	% of nests with more than 1 sp. of Zyg.
*Aphaenogaster***	76	10.97	14 (2)	230	3.03	8.24	5	6.6
Bothriomyrmex	2	0.29	2 (2)	11	5.50	0.50	0	–
*Camponotus***	87	12.55	12 (3)	243	2.79	5.17	7	8.0
Cataglyphis	13	1.88	3 (1)	59	4.54	45.77	1	8.3
Crematogaster	9	1.30	3 (–)	20	2.22	2.19	0	–
*Formica***	41	5.92	4 (1)	121	2.95	16.60	2	4.9
Goniomma	1	0.14	1 (–)	1	1.00	–	0	–
Lasius	19	2.74	1 (–)	56	2.95	12.72	0	–
Linepithema	3	0.43	6 (1)	20	6.67	65.33	0	–
*Messor***	331	47.76	16 (3)	2941	8.89	105.59	110	33.2
*Pheidole***	53	7.65	4 (4)	111	2.09	1.74	3	5.7
Plagiolepis	2	0.29	1 (1)	3	1.50	0.50	0	–
Tapinoma	7	1.01	2 (1)	11	1.57	1.29	0	–
Temnothorax	5	0.72	4 (1)	17	3.40	11.30	1	20.0
*Tetramorium***	44	6.35	7 (1)	155	3.52	16.44	3	7.1
Total	**693**	**100.00**	**19(7)**	**3999**	**5.77**	**64.54**	**132**	**19.0**

Tab. 6: Results of the tests for comparing the number of specimens per nest (grey background) and the proportion of nests with more than one species of Zygentoma (white background) that were found with the six most common genera of Formicidae hosting silverfish. In each cell, the value corresponds to the comparison between the Formicidae genera in the corresponding row and column. The number of specimens per nest was compared using the General Linear Model (family quasipoisson, link logarithmic, variance proportional to the square of the mean) and Bonferroni multiple comparison test. The proportion of nests with two or more species of silverfish was compared using the χ^2 test (Bonferroni correction applied). The multiple comparison of means statistic and its p-value are indicated in each test, and significant differences are marked with asterisks. Abbreviations used for ant genera are shown in Table 2.

Aph	0.0033	0.0979	321.0525	0.0464	0.0132	χ^2
	1.000	1.000	<0.01 ***	1.000	1.000	**p-value**
-0.0802	**Cam**	0.0976	321.0523	0.0462	0.0129	
1.000		1.000	<0.01 ***	1.000	1.000	
-0.0251	0.0551	**For**	321.1469	0.1408	0.1075	
1.000	1.000		<0.01 ***	1.000	1.000	
1077.041	1157.233	1102.168	**Mes**	321.0954	321.0622	
<0.01 ***	<0.01 ***	<0.01 ***		<0.01 ***	<0.01 ***	
-0.3681	-0.2879	-0.3430	-1445.148	**Phe**	0.0561	
1.000	1.000	1.000	<0.01 ***		1.000	
Estimate	0.1519	0.2321	0.1770	-0.9252	0.5200	**Tet**
p-value	1.000	1.000	1.000	<0.01 ***	1.000	

ic support because of the low number of samples. A bar chart of preferences was also presented in MOLERO-BALTANÁS & al. (1998).

Moreover, if the preferences of the category of occasional myrmecophiles (such as those of the genus *Lepisma*) are considered, it can be observed that they also show generalist trends. In fact, the correspondence analysis places them closer to generalist than to specialist species (Fig. 9).

Specialist silverfish. The remaining strict myrmecophiles can be assigned to the group of specialists. Apart from *Tricholepisma indalica* MOLERO-BALTANÁS, BACH DE ROCA & GAJU-RICART, 1995 which was found only on a few occasions with *Camponotus sylvaticus* (OLIVIER, 1792), they can be included in the following subdivisions:

• *Aphaenogaster* specialists: *Neoasterolepisma delator* MOLERO-BALTANÁS, BACH DE ROCA & GAJU-RICART, 1996 and *N. hesperica* MOLERO-BALTANÁS, BACH DE ROCA & GAJU-RICART, 1996 (Fig. 8) are found mostly with ants of the genus *Aphaenogaster*. Data in the literature, especially the numerous samples from Portugal that were reported by MENDES (1992, 2002), support this. The correspondence analysis places *Aphaenogaster* specialists far from *Messor* specialists and closer to generalist species (Fig. 9). Finally, despite the preferences of *N. pallida* MOLERO-BALTANÁS, GAJU-RICART & BACH DE ROCA, 1995 are quite marked, this species can be included in a group with intermediate characteristics. The correspondence analysis places it between generalist species and *Aphaenogaster* specialists (Fig. 9).

• *Messor* specialists: This constitutes the largest group within the Iberian fauna because eight species of *Neoasterolepisma* and one of *Tricholepisma* show strong preferences (often close to 100%) for ants of the genus *Messor* (see Tab. 2 and an example in Fig. 8). This group is clearly supported by the correspondence map (Fig. 9), which places *Messor* specialists very close together and separates all of them from the remaining Zygentoma.

Evolutionary relationships in Lepismatinae

The cladogram of Figure 10 includes Iberian and Canarian species in order to understand the origin of myrmecophily in Lepismatinae (see Material and methods section and Appendix S3). The cladogram shows that *Messor* specialists are the most apomorphic species of the group, and that generalist species and mainly the occasional myrmecophiles occupy the root of the tree.

Analysing data for detecting different degrees of tolerance of the Formicidae hosts

While the previous section is focused in the "silverfish point of view", in this section we centre on the Zygentoma-Formicidae relationship in terms of the ants. Tables 2 and 5 summarise the information of our samplings in Spain upon grouping the data by genus of Formicidae.

Considering the percentage of nests of the different ant genera harbouring Zygentoma, almost 50% of the total of ant nests corresponded to *Messor* (Fig. 2). Other genera with significant percentages are *Aphaenogaster* (10.5%), *Camponotus* (12.8%), *Formica* (6.2%), *Pheidole* WESTWOOD, 1839 (7.8%) and *Tetramorium* MAYR, 1855 (6.3%).

Table 5 shows data of all of these genera. The parameters that are compared include the number of species hosted by the different ant genera, the number of individuals that were found per nest (results in Tab. 6) and the number (and percentage) of nests in which two or more species of Zygentoma have been found (results in Tab. 6). Moreover, Table 7 shows the percentage of interactions of each genus of ants with specialist or generalist species of Zygentoma.

The three last parameters have been compared only in the genera of Formicidae with a significantly high number of samples. The GLM quasipoisson option has been followed by comparing the means of the number of silverfish

Tab. 7: Percentages of interactions with generalist or specialist silverfish in the nests of the most abundant genera of ants. The occasional myrmecophiles are considered generalists. Ant genera with fewer than ten interactions and the silverfish species *Tricholepisma indalica* (found only twice) are discarded. *Messor* is marked with bold characters to remark the clearly different tendency of this genus.

	Interactions with generalist Zygentoma	Interactions with specialist Zygentoma
Aphaenogaster	62.33%	37.67%
Camponotus	86.96%	13.04%
Cataglyphis	92.31%	7.69%
Formica	95.24%	4.76%
Lasius	89.47%	10.53%
Messor	**19.30%**	**80.7%**
Pheidole	98.21%	1.79%
Tetramorium	96.56%	4.44%

per nest because the variance, as a general rule, is nearer the square mean than the mean.

All of these comparisons show that the genus *Messor* produces very different results from the others: This genus harbours a clearly greater number of individuals than the other ant genera, the incidence of parabiosis is significantly higher in *Messor* samples and the majority of ant genera interact with generalist silverfish, except *Messor*, which interacts mostly with specialists.

Moreover, Tables 2 and 5 show how *Messor* harbours a higher diversity of silverfish. The number of species that are hosted in *Messor* nests is 16 (12, if those that were found just once are not taken into account), and this number can increase in more than 20 species if the whole Western Palaearctic is considered. The only genera showing a similar degree in the Iberian area are *Aphaenogaster* and *Camponotus*, which host 14 and 12 species, respectively (only 9 and 8 if species which were found only once were discarded). Nevertheless, *Camponotus* nests, as a whole, have a lower diversity because only associations with two generalist species are frequent (the remaining 10 species are collected rarely). This contrasts with *Messor* nests, where none of the proportions of their guests prevail (Fig. 11); the differences among hosted species may correspond to their higher or lower abundance or geographic range, presented in MOLERO-BALTANÁS & al. (2002).

The correspondence analysis (Fig. 9) also clearly separates *Messor* colonies from the remaining genera.

The ecological network Formicidae-Zygentoma

A graphic of the bipartite network representing the association of ant genera and silverfish species in Peninsular Spain and the Balearics is presented (Fig. 12). As described in other ecological bipartite networks, such as those presented by JORDANO & al. (2003) or BASCOMPTE & al. (2007), the silverfish-ants symbiotic network is very heterogeneous (most species have a few associations, but a few species are much more connected than is expected by chance), nested (specialists interact with subsets of the species with which generalists interact), and built on weak and asymmetric links among species.
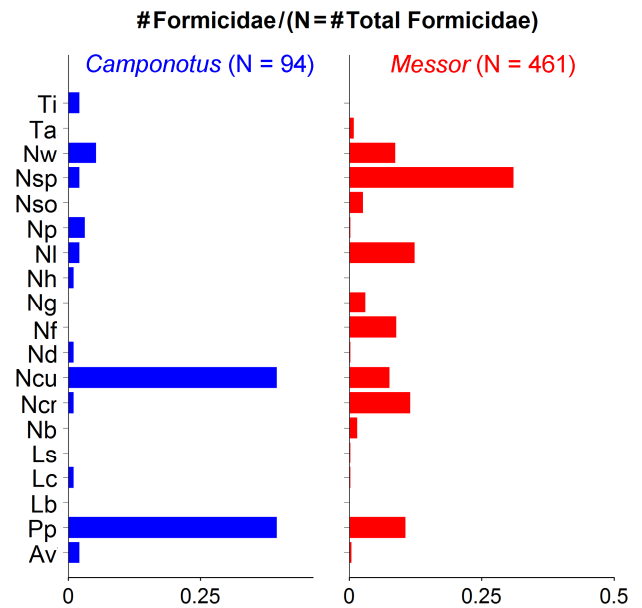
#Formicidae/(N=#Total Formicidae)

Fig. 11: Bar chart showing the proportions of association of *Camponotus* and *Messor* with different species of Zygentoma.

Tab. 8: Network metrics calculated for the Zygentoma-Formicidae network, with silverfish and ants considered at species level and pooling ants by genus.

Network metrics	Species level network (Tab. S2.1 in Appendix S2)	Network with ants pooled by genera (Tab. 2)
Connectance	0.1915	0.2970
Web-asymmetry	0.4242	-0.1515
Links per species	2.5910	2.3940
H2'	0.3081	0.3568
Modularity Q	0.3615	0.3931
Nestedness (temperature)	11.2225	8.6112

Some metrics of the network at the species level and the generic level (ants pooled by genus) are presented in Table 8. As *Messor* frequencies in the matrix do not reflect their relative abundance in the field because they are biased by the strong preferences of their specialists, d' and H2' are not the most relevant indices to take into account for a measure of specialisation (of single species and of the network as a whole, respectively). Both metrics are replaced by species-specificity index (SSI) and by modularity Q (DORMANN & STRAUSS 2014) as defined by R bipartite package (DORMANN & al. 2008). The first index (Tab. 4) gives values for each silverfish species that agrees with our classification criterion given in Table 1. Regarding the Q index of modularity, it shows a high value compared with most networks where this parameter has been calculated. Four modules can be detected by the QuaBiMo algorithm included in R bipartite package; from better to less-defined, these modules are: a) *Messor* and their specialists; b) *Aphaenogaster* and their specialists; c) most ant genera (mainly small ants) associated with the generalist Atelurinae and occasional *Lepisma*; d) common ant genera (especially, large ants) with the generalist *Neoas-*
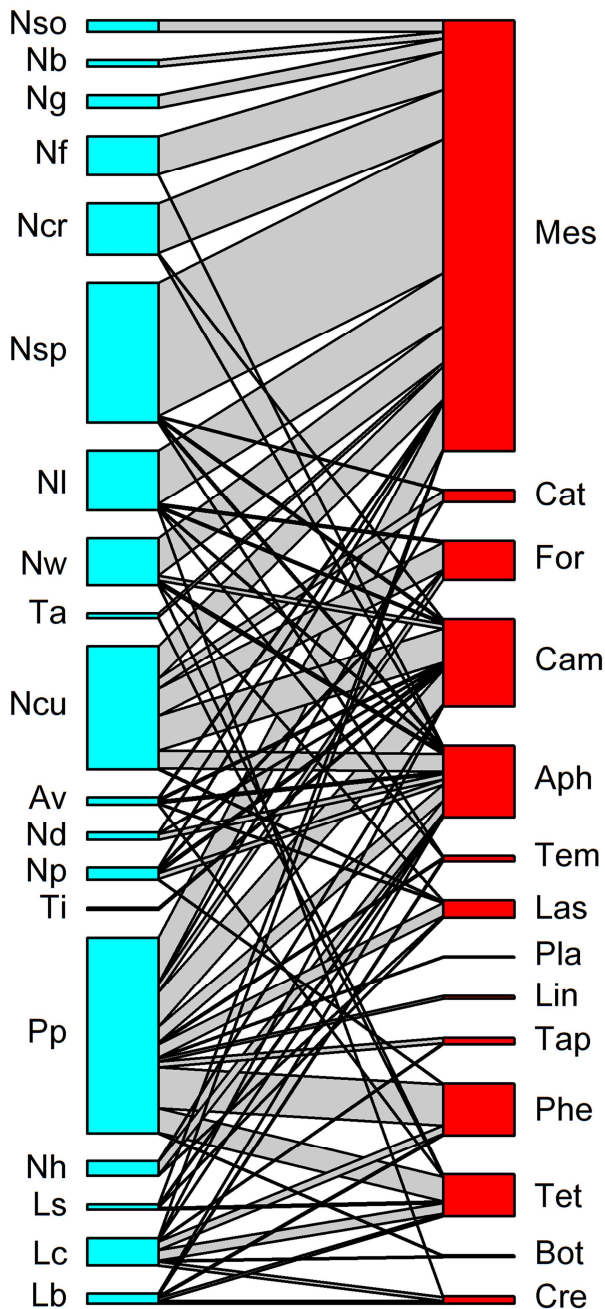
Fig. 12: Bipartite network Zygentoma species (left) – ant genera (right) in continental Spain. The graphic has been obtained by the R bipartite package from data of Table 2. The width of each link is proportional to the number of associations established between the connected taxa. Acronyms see Table 4.

terolepisma curtiseta and *N. pallida*. These modules can be seen in the plot of the correspondence analysis (Fig. 9).

Discussion

Classification of Zygentoma based on their preferences and their evolutionary trends

If we consider the data in the literature about Zygentoma-ant interactions over the entire Western Palaearctic region, several species can be added to the groups of myrmecophiles that have been distinguished.

Occasional myrmecophiles. There are some species of *Neoasterolepisma* in the Macaronesian region and North Africa that have been found only without ants or can be considered occasional myrmecophiles. This is the case of the Canarian species *Neoasterolepisma myrmecobia* (SILVESTRI, 1908), *N. vulcana* MENDES, BACH DE ROCA & GAJU-RICART, 1993 and *N. inexpectata* MENDES, MOLERO-BALTANÁS, BACH DE ROCA & GAJU-RICART, 1993, which have been included in the cladogram of Lepismatinae and are near the base of the tree (Fig. 10). This contrasts with our analysis on *Neoasterolepisma* based on Iberian species (Tab. 3), which classifies this genus, as a whole, as a Strict Myrmecophile. It seems that primitive species of the genus *Neoasterolepisma* were not myrmecophilous and that the relationship with ants developed after the origin of this clade.

Occasional myrmecophiles, such as these primitive *Neoasterolepisma* and *Lepisma chlorosoma*, are generalists (SSI index lower than 0.5). Our data suggest that they seem to prefer small-sized Myrmicinae (*Tetramorium*, *Pheidole*, and *Crematogaster* LUND, 1831).

Generalist species. The myrmecophilous relationships of the two Iberian Atelurinae have been discussed in MOLERO-BALTANÁS & al. (1998). *Proatelurina pseudolepisma* stands out from the rest of European myrmecophiles due to the width of its preferences (Tabs. 2, S2.1 in Appendix S2 and the histogram of Fig. 11 in MOLERO-BALTANÁS & al. 1998). The literature indicates that *Atelura formicaria*, which is widespread in Europe, is also a generalist species (PACLT 1963). Although the wide preferences of these species, we do not believe it appropriate to designate them as "panmyrmecophiles" because they are most likely not admitted throughout the Formicidae that have been reported in the area. A certain preference for Formicidae of small size as *Pheidole pallidula* (NYLANDER, 1849) has been observed, but large ants of the genus *Camponotus* are also frequent hosts of Spanish Atelurinae. The number of specimens per nest is usually low (1 - 3), but some *Pheidole* colonies host a higher number.

The bar chart of preferences of *Neoasterolepisma curtiseta* (Fig. 8) and the low SSI index are also typical of a generalist species: This silverfish has been found with high frequencies in the nests of several genera of ants (*Messor*, *Formica*, and *Camponotus* nests are the more visited). Although it represents a high percentage of *Formica* guests, this does not mean that *N. curtiseta* prefers *Formica*; this genus seems to be little attractive for silverfish and it is only visited occasionally by some generalist species.

Specialist myrmecophiles. In Spain, all species classified in this group belong to the subfamily Lepismatinae: They exhibit a limuloid shape, with a wide thorax due to the extension of lateral areas of nota (most likely to avoid the biting of the ants); most of them show golden scales, elongated tenth urotergite (particularly in females, most likely to protect the ovipositor) and shortened terminal filaments. Host-specific species also exhibit different types of apomorphic characters, such as a trend to sexual dimorphism concerning the shape and chaetotaxy of the hind tibiae of males, whose function is not clearly established (this setation is lacking in most generalists).

These characteristics can also be observed in species outside the Spanish fauna, such as *Tricholepisma gyriniformis* (LUCAS, 1846) from the South and East Mediterranean

region (a specialist in *Aphaenogaster*), the *Messor* specialist *Neoasterolepisma balcanica* (STACH, 1922) from the Eastern Mediterranean and some North African species such as *N. imitans* (MENDES, 1988). The *Messor* specialists group seems to be more diverse than the *Aphaenogaster* specialists, and also more widely distributed. Parabiosis is frequent in this group of specialists and deserves further study.

Evolutionary relationships among groups of Lepismatinae

The cladogram of Spanish Lepismatinae (Fig. 10) suggests that in the Western Palaearctic, silverfish of this subfamily began as occasional myrmecophiles with wide preferences (generalists) and then evolved to strict myrmecophiles. Specialists require a higher degree of morphological specialisation than generalists, and the cladogram places specialists as the more apomorphic clade. Inside these, species that are linked to *Messor* ants seem to be those that have acquired the most specialised condition.

Lepismatinae is an ancient group (the outgroup of the cladogram, *Allacrotelsa* SILVESTRI, 1935, is a xenomyrmecophile genus that occurs on both sides of the Atlantic Ocean), but it is not known exactly when Lepismatinae began to enter ant nests. It is likely that the beginning of this association befell more than 40 myr ago (after the break-up of Gondwanaland, since myrmecophile Lepismatinae are absent from the New World). As the genus *Messor* originated about 10 myr prior to *Aphaenogaster*, according the phylogeny of ant genera provided by MOREAU & al. (2006), we think that Lepismatinae living with *Messor* had more time to develop morphologic and ethologic specialisations to integrate with their hosts than *Aphaenogaster* specialists, which are most likely less integrated (R. Molero-Baltanás & M. Gaju-Ricart, unpubl.).

Are Zygentoma-Formicidae associations homogeneous? Analyses of data from associations in Spain suggest different levels of integration

As a consequence of the comparison of ant genera shown in Tables 5 - 7 (and also the data of the literature that confirm the same conclusions for the whole Western Palaearctic, see Appendix S1), three groups of Formicidae can be established according to their tolerance and specificity for the Zygentoma guests:

The first group includes ants with High and Broad Tolerance: genus *Messor*. These ants harbour a great number of species, several of which often cohabit in the same nest. The higher percentages of interactions correspond to specialist guests. Most of the Lepismatinae specialists are associated with *Messor*. The number of specimens found in each nest is comparatively high (and the actual number is much greater than the mean data shown in Table 5 because all of the specimens present could not be collected in many cases).

The second group of ants includes *Aphaenogaster*, with Low and Broad Tolerance. This genus harbours a high number of species, although the number of specimens per nest is usually low. The percentage of specialists that are lodged is not as high as that in *Messor* nests. Therefore, it is rare for two or more silverfish species to cohabit in *Aphaenogaster* nests. In contrast to what happens with *Messor* specialists, *Aphaenogaster* specialists seem to be mutu-

ally exclusive (*Neoasterolepisma delator*, *N. hesperica* and even *N. pallida* do not overlap in their geographical distributions but seem to be vicariants).

Finally, a third group of ants with a Low and Narrow Tolerance includes the remaining genera of Formicidae which harbour Zygentoma in their nests: *Camponotus*, *Formica*, *Pheidole*, *Cataglyphis* FÖRSTER, 1850, *Tetramorium*, etc. These ants permit the presence of few species of Zygentoma. Although *Camponotus* ants show more species, two generalist guests amount to more than the two-thirds of the total associations. The number of admitted specimens per nest is very low and, on rare occasions, two or more species of Zygentoma are found cohabiting in the same colony. Specialist Zygentoma have not been detected, unless *Tricholepisma indalica* could be allied with *Camponotus*.

The aforementioned differences among the different groups of ant genera could be related to different modalities of symbiotic relationship. At least two modes of association can be found inside West Palaearctic silverfish and ants; one of these modes can be interpreted as a trophic parasitism called "kleptoparasitism" and the other is most likely a case of commensalism, and can be designated "kleptobiosis". The difference between both terms was presented, for example, by IYENGAR (2008) or VOLLRATH (1984); kleptobionts steal items that have not been digested or used by the hosts or items that are so abundant that the consumption of a few of them by the guests does not incur an energetic cost to the host.

To understand and discuss the differences that are indicated by our results, it is necessary to compare several factors. The first one is the colony size. In regard to this, we could come to think that large colonies provide more abundant resources for silverfish (food and refuge). The available information about the colony size of *Messor* in Spain (BALLESTA & al. 1995) and France (CERDAN 1989) agrees with the classification by BARONI-URBANI (1977) in a group of ants with a moderately high colony size, except large nests of *M. barbarus*, with up to 23,000 workers, which can be classified in the high colony size group; this could explain our results (*Messor*, and especially *M. barbarus*, host a lot of species and individuals in the same nest). However, colonies of other genera in Spain are as large as *Messor* nests or larger, as indicated, for example, by data provided by BOULAY & al. (2007) on *Aphaenogaster* or by HUAN & DORNHAUS (2008) on *Formica*; silverfish inhabit them significantly less. Thus, we think that the colony size is not the key factor that explains the differences between *Messor* and the remaining common genera. This factor can be used only to justify that genera such as *Temnothorax* MAYR, 1861, classified by BARONI-URBANI (1977) in a group with small nests and low biomass / number or workers, show a lower number and diversity of silverfish.

If we focus on the different levels of aggressiveness as a factor to explain our differences, it could be concluded that the most aggressive ants should be those with less diversity and fewer guests, which could imply that *Messor* are the less aggressive ants of the Iberian fauna. However, if they were less aggressive, the frequencies of generalist silverfish could be higher in *Messor* than in other ants, but this is not what happens, as shown in the bar charts of Atelurinae and *Neoasterolepisma curtiseta*

(Fig. 8). The available information to grade the relative levels of aggressiveness of the different taxa of ants is based on "ant against ant" aggression tests (Retana & Cerdá 1995, Cerdá & al. 1997, etc.); from these data, it is clear that *Messor* are far from being the least aggressive ants. However, no data are available regarding "ant against silverfish" tests.

Zygentoma are preadapted to run, so the quick escape is the more plesiomorphic of their strategies to avoid aggression. A second strategy of myrmecophile species is chemical mimicry: The available studies on this topic (Witte & al. 2009, Lenoir & al. 2012) indicate that silverfish acquire the odour of the ants by contact. This behaviour has been probably developed only by the more specialised species. Most Zygentoma that are considered occasional or generalist myrmecophiles probably use only the ancestral escape strategy and often prefer small ants (with small mandibles), as supported by our data. *Messor* specialists and perhaps some generalists can live with large ants (potentially more harmful for silverfish) because they have developed the behaviour of approaching workers to acquire their odour, managing to scrape the ventral part of the body of the host; this strategy is favoured by the large size of the ant.

The more relevant factor that explains our results (mainly the differences between *Messor* and the remaining ant genera) is surely the diet of the ants. Most Western Palaearctic ant genera of Formicidae generate a small volume of residues that are available to the Zygentoma; in these colonies, silverfish probably develop a more primitive strategy of trophic parasitism (kleptoparasitism), stealing the food that has been pre-digested by the ants and attempting to go unnoticed. In this case, the presence of a reduced number of parasites in the nest (shown by our results) could be advantageous. Conversely, in *Messor* colonies, the abundant nutritional resources that these ants generate attract a lot of guests and favour the evolution of numerous specialists that have adapted to live in these nests. Although a wide range of silverfish guests is also observed with *Camponotus* and *Aphaenogaster*, these genera of ants host few specialists. We think that *Messor* is different because it seems to have established a more "friendly relationship" with Lepismatinae specialists. This association is most likely related to the seed-based diet of the ants and the possibility of a cleaning symbiosis (mutualistic association) or at least of a "welcome" kleptobiotic commensalism. As Mendes (1987) had already suggested, the relationship between *Messor* and their guests is based on a nutritional complementarity: Ants are unable to digest the husks of the gathered seeds (these husks must be expelled from the nest) and Lepismatinae silverfish (but probably not Atelurinae) are capable of feeding on these and other residues. The capacity to digest cellulose and other carbohydrates without the need for endosymbiotic microbiota is very uncommon among insects, but has been demonstrated in some species of Lepismatidae (Lasker & Giese 1956). If this feeding complementarity of *Messor* and their specialists exists, the high density of the population of silverfish in the nest is not an important problem for the ants. As the cladogram of Figure 10 demonstrates, *Messor* specialists are the more evolved inside myrmecophilous Lepismatinae, developing strategies to achieve a higher level of integration with their hosts.

Network interpretation

A detailed interpretation of the Zygentoma-Formicidae network represented in Figure 12 is not the aim of this work, but some aspects of the association and the sampling in which it is based should be taken into account for further in-depth analysis:

As it has been proved (Dormann & Strauss 2014), a high value of modularity Q is correlated with high H2' levels, implying high levels of specialisation. According to Blüthgen & al. (2006), it can be interpreted that the low values of H2' calculated are due to an overestimation of *Messor* abundance and not to low levels of specialisation.

The geographic area from where the data are obtained is very large, about 400,000 km^2, and some different types of ecosystems are included.

Some silverfish species are not widespread over the entire study area (some of them are endemic of a relatively reduced area and even some of them can be considered as vicariants), but treated as genera, the distribution of most ants covers the whole area.

As argued before, this is not a homogeneous network in terms of the type of association. It is likely that a good number of the relationships between occasional or generalist Zygentoma and ants are antagonistic, but indications are presented that associations between *Messor* specialists and their hosts tend to be of commensalism or even mutualistic. The best way to represent these associations is probably a merged tripartite network, such as those presented by Sauve & al. (2014). Ant genera can be placed in a central column, parasite silverfish in a second column on one side and commensals or mutualistic guests can be included in a third column on the contrary side. In Spain, exclusively all *Messor* specialists can be included in this column. Nevertheless, this positive relationship is not comparable to typical mutualistic plants-pollinators networks, where there is an important (nearly symmetric) mutual dependence. *Messor* ants probably do not depend significantly on silverfish, because the benefit they obtain is not indispensable. The relationship is very asymmetric and coevolution processes are not clearly developed. Silverfish have not significantly conditioned the evolution of ants, but Lepismatinae silverfish have experienced important adaptive modifications (morphological, biological and ethological) to live inside ant colonies, and the more striking modifications seem to be developed in *Messor* specialists, meaning that this group deserves further investigation.

Conclusions

Here, 157 associations between Zygentoma and Formicidae species are reported in Spain, with 41 of them described for the first time. Adding our data to those of the literature, 193 different associations are known in the Western Palaearctic region.

Spanish silverfish species are classified according to their preferences for ants into four groups: Xenomyrmecophiles, Occasional Myrmecophiles, Strict Generalist Myrmecophiles and Strict Specialist Myrmecophiles. The higher number of species is inside this last group and most of them (9 species) are *Messor* specialists. This classification has a statistical and phylogenetic support.

Messor ants host a higher number of species and specimens per nest than the remaining genera of ants studied,

suggesting that the association with their specialist partners is different (probably, commensalism or even mutualistic) from those associations established between ants and generalist silverfish (antagonistic). The seed-based diet of these ants could be the main factor influencing the evolution of a group of silverfish species with a higher level of integration.

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